

Calcium phosphate supersaturation regulates stone formation in genetic hypercalciuric stone-forming rats

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Background. Hypercalciuria is the most common metabolic abnormality observed in patients with nephrolithiasis. Hypercalciuria raises urine supersaturation with respect to the solid phases of calcium oxalate and calcium phosphate, leading to an enhanced probability for nucleation and growth of crystals into clinically significant stones. However, there is little direct proof that supersaturation itself regulates stone formation. Through successive inbreeding of the most hypercalciuric progeny of hypercalciuric Sprague-Dawley rats, we have established a strain of rats, each of which excrete abnormally large amounts of urinary calcium and each of which forms calcium phosphate kidney stones. We used these hypercalciuric (GHS) rats to test the hypothesis that an isolated reduction in urine supersaturation, achieved by decreasing urine phosphorus excretion, would decrease stone formation in these rats.

Methods. Thirty 44th-generation female GHS rats were randomly divided into three groups. Ten rats received a high-phosphorus diet (0.565% phosphorus), 10 a medium-phosphorus diet (0.395% phosphorus), and 10 a low-phosphorus diet (0.225% phosphorus) for a total of 18 weeks. The lowered dietary phosphorus would be expected to result in a decrease in urine phosphorus excretion and a decrease in urinary supersaturation with respect to the calcium phosphate solid phase. Every two weeks, 24-hour urine collections were obtained. All relevant ions were measured, and supersaturation with respect to calcium oxalate and calcium hydrogen phosphate were determined. At the conclusion of the experiment, each rat was killed, and the kidneys, ureters, and bladder were dissected en block and x-rayed to determine whether any stones formed. A decrease in stone formation with a reduction in urinary supersaturation would support the hypothesis that supersaturation alone can regulate stone formation.

Results. Decreasing the dietary phosphorus intake led to a progressive decrease in urine phosphorus excretion and an increase in urine calcium excretion, the latter presumably caused by decreased intestinal calcium phosphate binding and increased calcium absorption. With decreasing dietary phosphorus intake, there was a progressive decrease in saturation

with respect to the calcium phosphate solid phase. Fifteen of the 20 kidneys from the 10 rats fed the high-phosphorus diet had radiographic evidence of kidney stone formation, whereas no kidneys from the rats fed either the medium- or low-phosphorus diet developed kidney stones.

Conclusions. A decrease in urine phosphorus excretion not only led to a decrease in urine supersaturation with respect to the calcium phosphate solid phase but to an elimination of renal stone formation. The results of this study support the hypothesis that variation in supersaturation alone can regulate renal stone formation. Whether a reduction of dietary phosphorus will alter stone formation in humans with calcium phosphate nephrolithiasis remains to be determined.

Hypercalciuria is the most common metabolic abnormality in patients with nephrolithiasis [1–5]. Hypercalciuria raises urine supersaturation with respect to the solid phases of calcium oxalate and calcium phosphate, enhancing the probability of nucleation and growth of crystals into clinically significant stones [2]. The reversal of the hypercalciuria with consequent lowering of supersaturation with thiazide diuretics has been shown to reduce clinical stone formation in two long-term studies [6, 7]. In addition to the hypocalciuric action of thiazide diuretics, these agents also reduce the efficiency of urinary osmotic concentration, perhaps playing a role in the prevention of stone formation. There is little direct proof that supersaturation itself regulates stone formation.

Through successive inbreeding of the most hypercalciuric progeny of hypercalciuric Sprague-Dawley rats, we have established a strain of rats, each of which excrete abnormally large amounts of urinary calcium [8–19]. The principal mechanism for the excessive calcium excretion in these rats appears to be an increase in intestinal calcium absorption [19]. The increased intestinal calcium absorption appears to be mediated not by an increase in the serum level of $1,25(\text{OH})_2\text{D}_3$, but by an increase in the number of intestinal vitamin D receptors [18]. When these hypercalciuric rats are fed a very low-calcium diet, their urine calcium excretion remains elevated compared with that of similarly treated control rats, indi-

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cating a defect in renal calcium reabsorption and/or an increase in bone resorption [17]. The bone from these hypercalciuric rats releases more calcium, compared with bone of control rats, when exposed to increasing amounts of $1,25(\text{OH})_2\text{D}_3$ [14], and inhibition of bone resorption substantially decreases the hypercalciuria when these rats are fed a low-calcium diet [8]. In addition, a primary defect in renal calcium reabsorption is observed during carefully controlled clearance studies [12]. We have shown that in addition to the intestine, both the bone and kidney of the hypercalciuric rats have an increased number of vitamin D receptors [10, 14, 18]. Thus, these hypercalciuric rats appear to have a systemic abnormality in calcium homeostasis. They absorb more intestinal calcium. They resorb more bone, and they fail to adequately reabsorb filtered calcium. As each of these hypercalciuric rats forms renal stones, we have termed the rats genetic hypercalciuric stone-forming (GHS) rats [9, 13, 15]. The stones formed contain only calcium and phosphate, without oxalate, and, by x-ray diffraction, are exclusively poorly crystalline apatite. Calcium transport abnormalities similar to those documented in the GHS rats have been observed in many patients with idiopathic hypercalciuria and nephrolithiasis [2].

In this study, we tested the hypothesis that an isolated reduction in urine supersaturation, achieved by decreasing urine phosphorus excretion, would decrease stone formation in our GHS rats. We fed the rats decreasing amounts of dietary phosphorus, which would be expected to result in less urinary phosphorus and a decrease in supersaturation. We found that a decrease in urine phosphorus excretion not only led to a decrease in urine supersaturation with respect to the calcium phosphate solid phase but to an elimination of renal stone formation. The results of this study support the hypothesis that variation in supersaturation alone can regulate renal stone formation and suggest the advisability of human studies employing phosphorus restriction in patients who form calcium phosphate kidney stones.

METHODS

Establishment of hypercalciuric rats

Adult Sprague-Dawley rats (Charles River Laboratories, Kingston, NY, USA) were screened for hypercalciuria. The rats were placed in individual metabolic cages and placed on a constant amount of a standard calcium diet, and the urine calcium excretion was measured. The most hypercalciuric male and female rats were used to breed the next generation. A similar protocol was used for screening and inbreeding of subsequent generations as described previously [8–19].

Study protocol

Thirty 44th-generation female GHS rats, each initially weighing 125 g, were placed in metabolic cages for a

total of 18 weeks. Each rat was offered 13 g/day of diet, an amount that we have previously shown is completely consumed by a female rat of this size [8]. A standard 1.2% calcium, 0.65% phosphorus diet was mixed with a 1.2% calcium, 0.14% phosphorus diet (produced by substituting chloride for most phosphorus in the standard diet) in a ratio of 5:1, 1:1, or 1:5, resulting in a 0.565% phosphorus diet (termed high phosphorus), a 0.395% phosphorus diet (termed medium phosphorus), and a 0.225% phosphorus diet (termed low phosphorus), respectively. Our goal was to decrease progressively dietary phosphorus below that of the standard diet, which we have previously shown results in stone formation in 100% of GHS rats by 18 weeks [9, 13, 15].

The rats were randomly divided into three groups. Ten rats received the high-phosphorus diet, 10 the medium-phosphorus diet, and 10 the low-phosphorus diet. Every two weeks, two successive 24-hour urine collections were obtained. The lowered dietary phosphorus would be expected to result in a decrease in urine phosphorus excretion and a decrease in urinary supersaturation with respect to the calcium phosphate solid phase. The first 24-hour urine was collected in thymol and was used for all measurements except oxalate, and the second 24-hour collection was collected in concentrated HCl for measurement of oxalate. Both samples were refrigerated at 0°C, and biochemical measurements were determined within two weeks. At the conclusion of the experiment (18 weeks), each rat was killed, and the kidneys, ureters, and bladder were dissected en block and mounted on radiographic film. A decrease in stone formation with reduction in urinary supersaturation would support the hypothesis that supersaturation alone can regulate stone formation. Any rat that ate less than 12 g of food or drank less than 15 mL of water on any day of the study would have been excluded from the entire study; however, all rats met these prospective criteria throughout the study.

Chemical determinations

Calcium was measured by reaction with arsenazo III and was then determined photometrically at 650 nm [20]. Creatinine was determined by a modification of the Jaffe method by formation of a creatinine-picric acid complex [21]. Inorganic phosphorus was measured by reaction with ammonium molybdate to form a colored phosphomolybdate complex [22]. Uric acid was measured after oxidation by uricase to produce allantoin and hydrogen peroxide [23]. Magnesium was determined by combination with calmagite [24]. Ammonia was determined by coupled enzyme system using glutamate dehydrogenase and NADPH [25]. Sodium was determined by a selective electrode [26] and potassium using a valinomycin membrane attached to a potassium electrode [27]. Chloride was measured by colorimetry using a silver/silver chlo-

ride electrode [28]. Oxalic acid was measured using oxalate oxidase, which oxidizes oxalate to hydrogen peroxide and carbon dioxide. The hydrogen peroxide then reacts with 3-methyl-2-benzothiazolinone hydrozone and 3-(dimethyl)benzoic acid to form an indamine dye [29]. Citric acid was determined using citrate lyase, which catalyzes the conversion citrate to oxaloacetic acid, which is then converted to malic acid, in the presence of malate dehydrogenase. The malic acid oxidizes NADH to NAD⁺ [30]. pH was measured by an ion-selective electrode. Sulfate was measured by turbidity after barium precipitation [31].

Urinary supersaturation

The calcium oxalate ion activity product was calculated using the computer program EQUIL, which was developed by Finlayson [32–34]. The computer program calculates free ion concentrations using the concentrations of measured ligands and known stability constants. Ion activity coefficients are calculated from ionic strength using the Davies modification of the Debye-Huckel solution to the Poisson-Boltzman equation. The program simultaneously solves for all known binding interactions among the measured substances. Oxalate, phosphorus, and calcium ion activities were used to calculate the free-ion activity products. The free ions in solution are considered to be in an equilibrium with the dissolved calcium oxalate governed by a stability constant (*K*) of 2.746×10^3 mol/L and with the dissolved brushite governed by a *K* of 0.685×10^3 mol/L. The value of calcium oxalate in a solution at equilibrium with a solid phase of calcium oxalate, the solubility of calcium oxalate, is 6.16×10^{-6} mol/L. The value of the brushite in a solution at equilibrium with a solid phase of brushite, the solubility of brushite, is 3.981×10^{-7} mol/L. The relative supersaturation for calcium oxalate is calculated as the ratio of the free-ion activity product of calcium and oxalate in the individual urine to the solubility of calcium oxalate. The relative supersaturation for brushite is calculated as the ratio of the free-ion activity product of calcium and phosphate in the individual urine to the solubility of calcium phosphate. Ratios of 1 connote a sample at equilibrium, above 1 supersaturation, and below 1 undersaturation.

The ability of this computer program to predict accurately the saturation of urine or other solution with respect to the solid phase is excellent [16, 17, 32, 35, 36]. With a series of 20 artificial solutions, the equilibrium calcium concentration and the extent of calcium precipitation were predicted with average errors of $5 \pm 9\%$ and $5 \pm 8\%$ (mean \pm SD), respectively [32]. We have used this computer program previously and found excellent correspondence between calculated and experimentally measured saturations in urine and blood [8, 9, 11, 15–17] and in bone culture medium [35–37].

Statistical analysis

All values are expressed as mean \pm SE. Tests of significance were calculated by analysis of variance with the Bonferroni correction for multiple comparisons and regression analysis using conventional computer programs (BMDP; University of California, Los Angeles, CA, USA) on a digital computer. A *P* < 0.05 was considered significant.

RESULTS

Urinary ion excretion

Every two weeks, two successive 24-hour urine collections were obtained. The individual urine collections for the 30 rats divided equally into three groups were analyzed separately and were then averaged over the first six weeks (weeks 1 to 6), the second six weeks (weeks 7 to 12), and the final six weeks (weeks 13 to 18).

With respect to urine phosphorus, during each of the three individual six-week time periods, there was a decrease in urine phosphorus excretion in rats eating the medium-phosphorus diet compared with rats eating the high-phosphorus diet and a further decrease in urine phosphorus excretion in rats eating the low-phosphorus diet compared with rats eating the medium-phosphorus diet; rats eating the low-phosphorus diet excreted less phosphorus than those eating the high-phosphorus diet (Fig. 1, top). Thus, there was a progressive decrease in phosphorus excretion with decreasing dietary phosphorus intake.

With respect to urine calcium, during weeks 1 to 6 and 7 to 12, there was an increase in urine calcium excretion in rats eating the medium-phosphorus diet compared with rats eating the high-phosphorus diet, and a further increase in urine calcium excretion in rats eating the low-phosphorus diet compared with rats eating the medium-phosphorus diet; rats eating the low-phosphorus diet excreted more calcium than those eating the high-phosphorus diet (Fig. 1, middle). During weeks 13 to 18, although there was an increase in urine calcium excretion in rats eating the medium-phosphorus diet compared with rats eating the high-phosphorus diet, there was no change in urine calcium excretion in rats eating the low-phosphorus diet compared with rats eating the medium-phosphorus diet, and rats eating the low-phosphorus diet excreted more calcium than those eating the high-phosphorus diet. Thus, in general, there was a progressive increase in calcium excretion with decreasing dietary phosphorus intake.

With respect to urine oxalate, during each of the three individual six-week time periods, there was no difference in oxalate excretion between rats eating the high-, medium-, or low-phosphorus diet (Fig. 1, bottom). Thus, dietary phosphorus has no detectable effect on urine oxalate excretion.

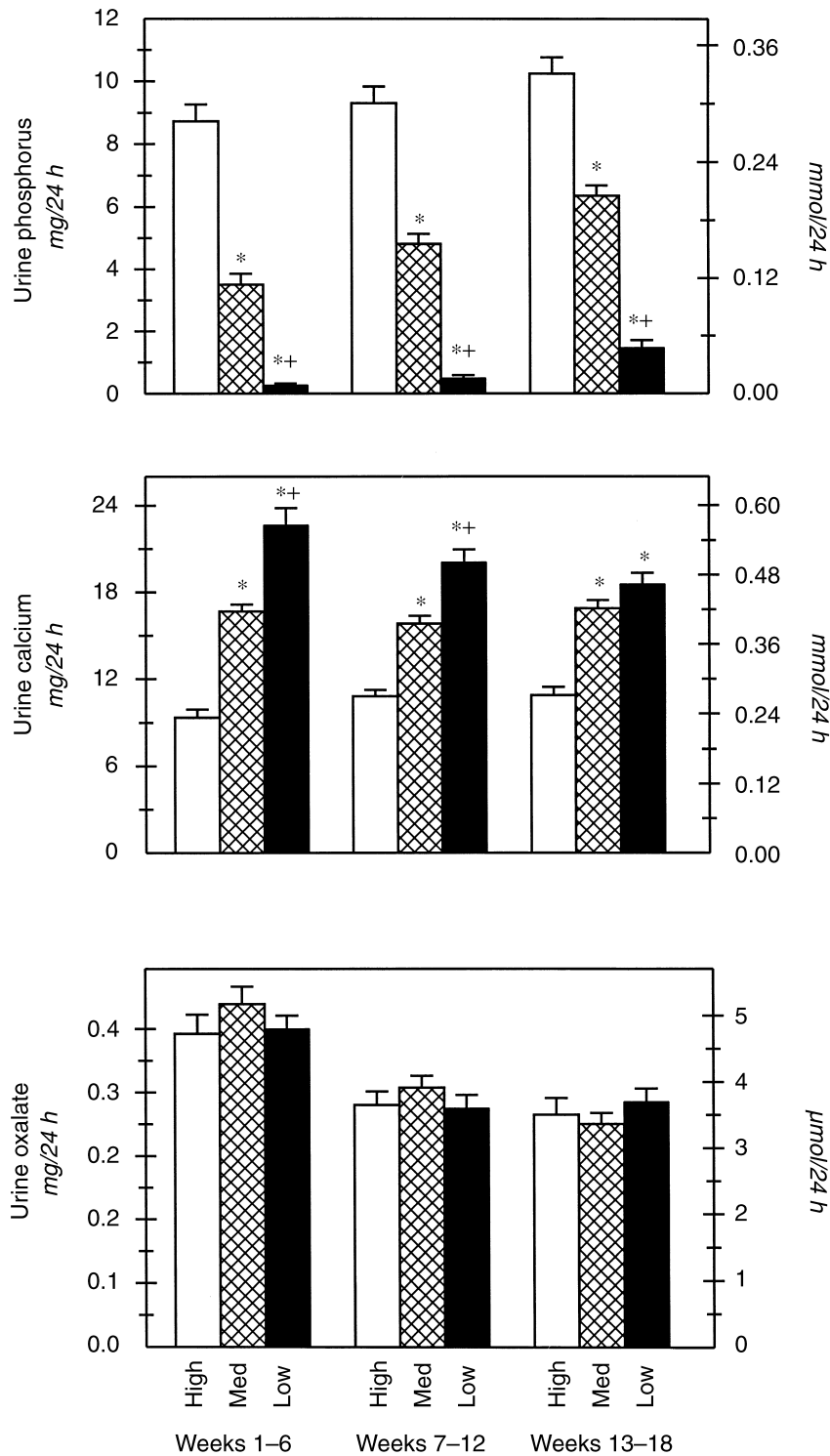


Fig. 1. Urine phosphorus, calcium, and oxalate excretion (mean \pm SE) in genetic hypercalciuric stone forming (GHS) rats fed 13 g/day of either a high-, medium-, or low-phosphorus diet for a total of 18 weeks. Every two weeks, two successive 24-hour urine collections were obtained. The individual urine collections for the 30 rats divided equally into three groups were analyzed separately and were then averaged over the first six weeks (weeks 1 through 6), the second six weeks (weeks 7 through 12), and the final six weeks (weeks 13 through 18). Abbreviations are as follows: High, GHS rats fed 13 g/day of a 0.565% phosphorus diet; Med, GHS rats fed 13 g/day of a 0.395% phosphorus diet; Low, GHS rats fed 13 g/day of a 0.225% phosphorus diet. *Different from High, $P < 0.05$; +Different from Med, $P < 0.05$.

With respect to urine magnesium, during each of the three individual six-week time periods, there was an increase in urine magnesium excretion in rats eating the medium- and low-phosphorus diet compared with rats eating the high-phosphorus diet, and there was no differ-

ence in urine magnesium excretion between rats eating the medium- and low-phosphorus diets (Fig. 2, top).

With respect to urine chloride, during each of the three individual six-week time periods, there was an increase in urine chloride excretion in rats eating the medium-

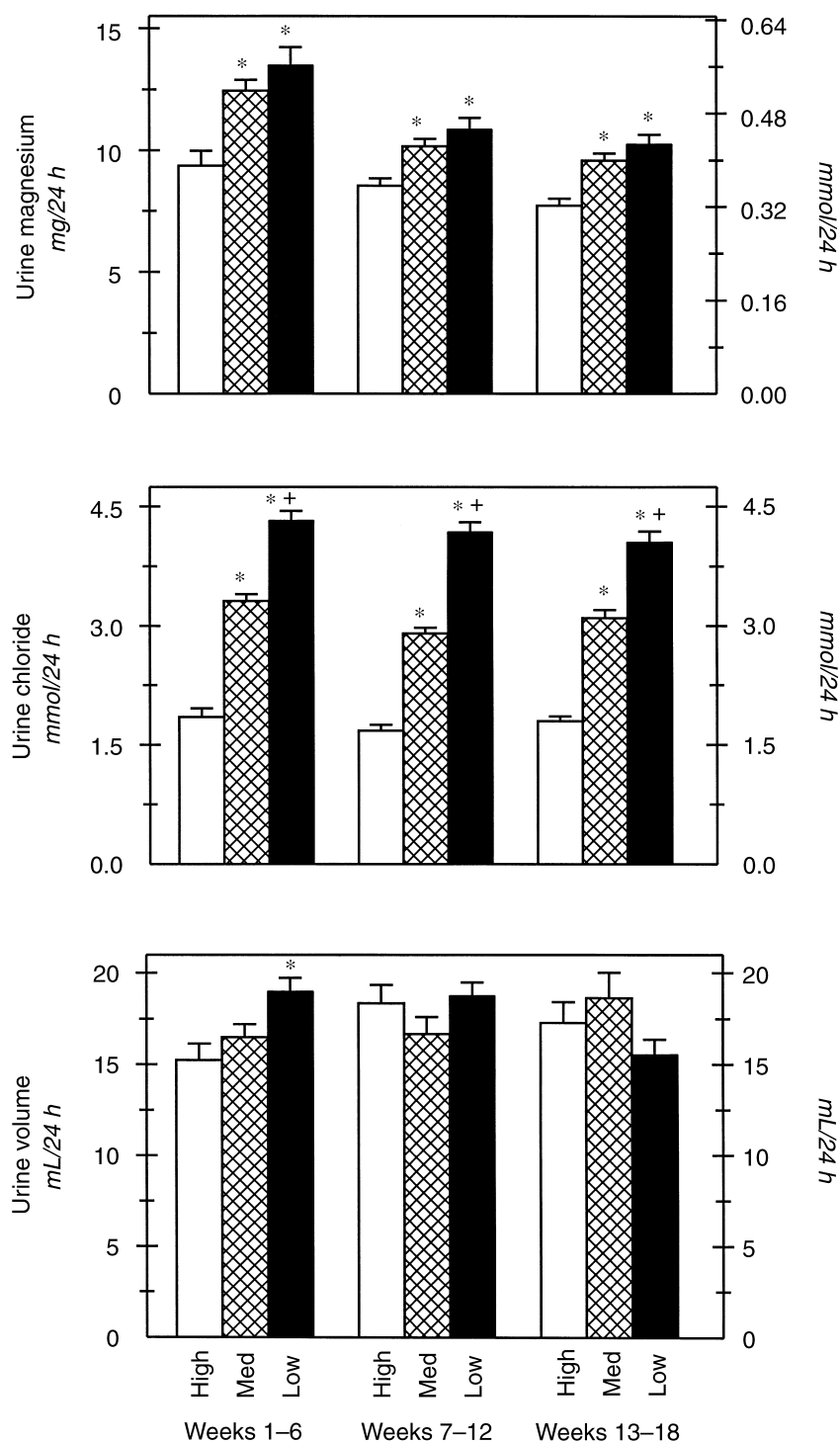


Fig. 2. Urine magnesium and chloride excretion and volume (mean \pm SE) in GHS rats fed 13 g/day of either a high-, medium-, or low-phosphorus diet for a total of 18 weeks. Methods and abbreviations are the same as in Figure 1.

phosphorus diet compared with rats eating the high-phosphorus diet, and a further increase in urine chloride excretion in rats eating the low-phosphorus diet compared with rats eating the medium-phosphorus diet. Rats eating the low-phosphorus diet excreted more chloride than those eating the high-phosphorus diet (Fig. 2, mid-

dle). Thus, there was a progressive increase in chloride excretion when dietary chloride was substitute for dietary phosphorus.

With respect to urine volume, during each of the three individual six-week time periods, there was no difference in urine volume between rats eating the low-, medium-,

or high-phosphorus diet, except that there was an increase in urine volume in rats eating the low compared with those eating the high-phosphorus diet during weeks 1 through 6 (Fig. 2, bottom).

With respect to urine sodium, during each of the three individual six-week time periods, there was no difference in sodium excretion between rats eating the high-, medium-, or low-phosphorus diet, except that there was an increase in urine sodium in rats eating the medium compared with the high-phosphorus diet during weeks 1 through 6 (data not shown). Thus, dietary phosphorus has little effect on urine sodium excretion.

With respect to urine pH, during weeks 1 through 6, there was an increase in urine pH in rats eating the low-phosphorus compared with those eating the medium-phosphorus diet (Fig. 3, top). During weeks 7 through 12, there was a decrease in urine pH in rats eating the low-phosphorus diet compared with those eating the high- and medium-phosphorus diet, and during weeks 13 through 18, there was a decrease in urine pH in rats eating the medium- and low-phosphorus diet compared with those eating the high-phosphorus diet.

With respect to urine citrate, during each of the three individual six-week time periods, there was a decrease in urine citrate in rats eating the low-phosphorus diet compared with those eating the high- and the medium-phosphorus diet (Fig. 3, middle). Additionally, during weeks 13 through 18, there was a decrease in urine citrate in rats eating the medium-phosphorus diet compared with those eating the high-phosphorus diet.

With respect to urine ammonium, during each of the three individual six-week time periods, there was an increase in urine ammonium excretion in rats eating the medium-phosphorus diet compared with rats eating the high-phosphorus diet, and a further increase in urine ammonium excretion in rats eating the low-phosphorus diet compared with rats eating the medium-phosphorus diet. Rats eating the low-phosphorus diet excreted more ammonium than those eating the high-phosphorus diet (Fig. 3, bottom). Thus, there was a progressive increase in ammonium excretion with decreasing dietary phosphorus intake.

Urinary supersaturation

With respect to urine CaHPO_4 (brushite) supersaturation, during each of the three individual six-week time periods, there was a decrease in urine CaHPO_4 supersaturation in rats eating the medium-phosphorus diet compared with rats eating the high-phosphorus diet, and a further decrease in urine CaHPO_4 supersaturation in rats eating the low-phosphorus diet compared to rats eating the medium-phosphorus diet. Rats eating the low-phosphorus diet had a lower urine CaHPO_4 supersaturation compared with those eating the high-phosphorus diet (Fig. 4, top). Thus, there was a progressive decrease in

CaHPO_4 supersaturation with decreasing dietary phosphorus intake.

With respect to urine CaOx (calcium oxalate) supersaturation, during weeks 1 through 6, there was a decrease in CaOx supersaturation in rats fed the low-phosphorus diet compared with the medium-phosphorus diet (Fig. 4, bottom). During weeks 7 through 12, there was an increase in CaOx supersaturation in rats fed the medium-phosphorus compared with the high-phosphorus diet and a decrease in rats fed the low-phosphorus compared with the medium-phosphorus diet. During weeks 13 through 18, there were no differences in CaOx supersaturation among the three groups.

Stone formation

Fifteen of the 20 kidneys from the 10 rats fed the high-phosphorus diet had radiographic evidence of kidney stone formation, whereas no kidneys from the rats fed either the medium or low-phosphorus diet developed kidney stones (Figs. 5 and 6). Ten of the 10 rats fed the high-phosphorus diet had radiographic evidence of kidney stone formation, whereas none of the rats fed either the medium- or low-phosphorus diet developed kidney stones.

DISCUSSION

Genetic hypercalciuric stone-forming rats spontaneously form kidney stones when fed a normal calcium (1.2%) and phosphorus (0.65%) diet [9, 13, 15]. In this study, we tested the hypothesis that reductions in dietary phosphorus would lead to reductions in urinary phosphorus excretion and calcium phosphorus supersaturation, leading to a fall in the number of stones formed. We found that although 75% of kidneys from GHS rats fed a 0.565% phosphorus diet contained kidney stones after 18 weeks, there was no stone formation in rats fed a 0.395% or a 0.225% phosphorus diet.

Quantitatively, the majority of intestinal phosphorus absorption occurs by paracellular diffusion, indicating that a decrease in dietary phosphorus should decrease intestinal absorption, decrease the filtered load of phosphorus, and decrease urine phosphorus excretion [38, 39]. However, decreasing dietary phosphorus will also lead to an increase in the rate of hydroxylation of $25(\text{OH})\text{D}_3$ -1, $25(\text{OH})_2\text{D}_3$, which will increase cell-mediated intestinal phosphorus absorption [40]. In this study, the net result of an decrease in dietary phosphorus led to a marked decrease in urine phosphorus excretion (Fig. 1, top). This is similar to findings in humans in which decreasing dietary phosphorus intake leads to an decrease in urine phosphorus excretion [41].

Alterations in dietary phosphorus should have important effects not only on urine phosphorus excretion, but on urinary calcium excretion. Dietary phosphorus and

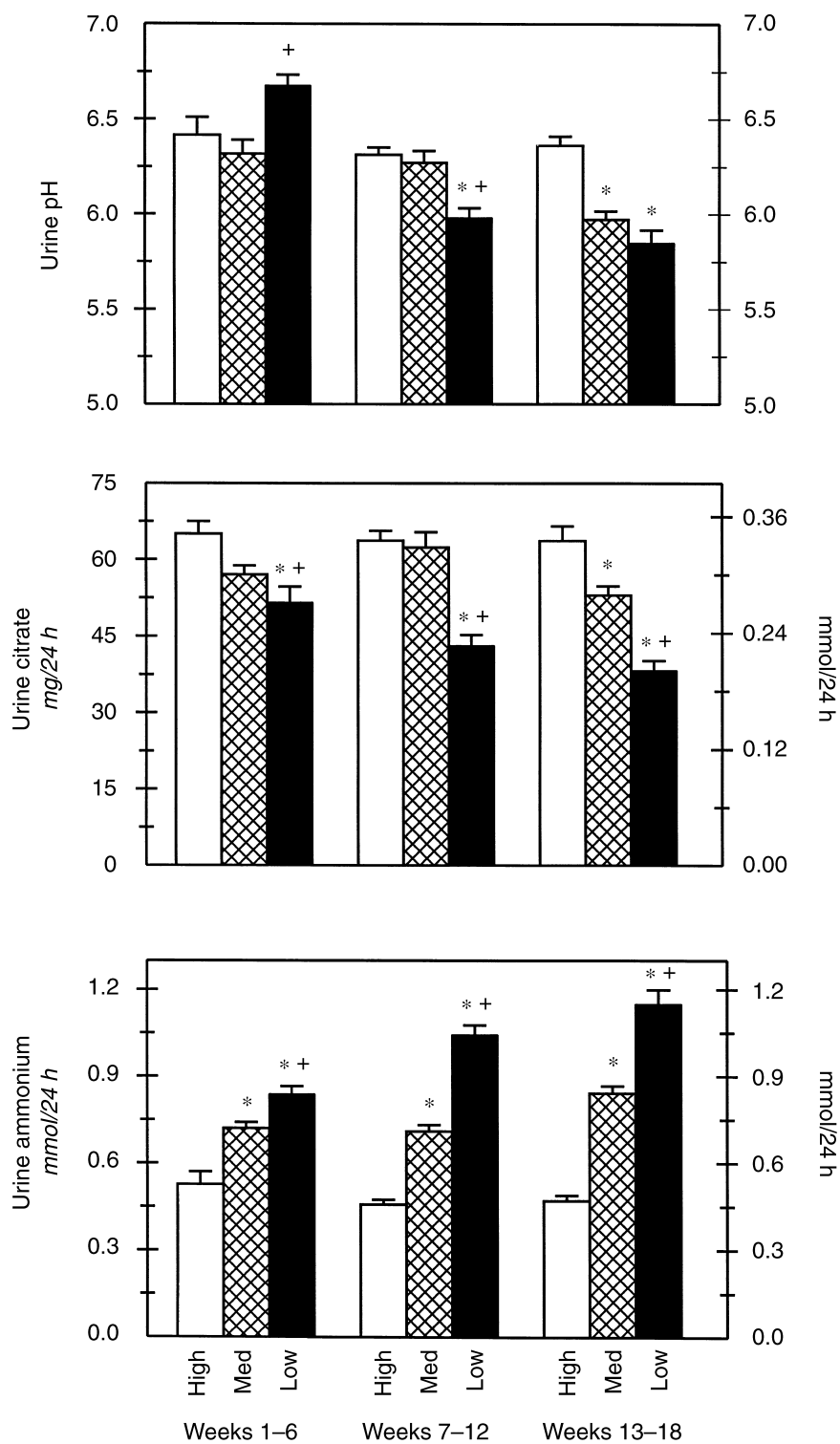


Fig. 3. Urine pH and citrate and ammonium excretion (mean \pm SE) in GHS rats fed 13 g/day of either a high-, medium-, or low-phosphorus diet for a total of 18 weeks. Methods and abbreviations are the same as in Figure 1.

calcium bind into an unabsorbable complex [42]. Thus, reductions in dietary phosphorus intake should result in an increase in calcium available for absorption and subsequent excretion. The phosphorus restriction induced increase in $1,25(\text{OH})_2\text{D}_3$ will also promote intesti-

nal calcium absorption leading to increased urine calcium excretion [43]. In the present study, a decrease in dietary phosphorus led to an increase in urine calcium (Fig. 1, middle). There was a strong inverse correlation between urine phosphorus and urine calcium excretion,

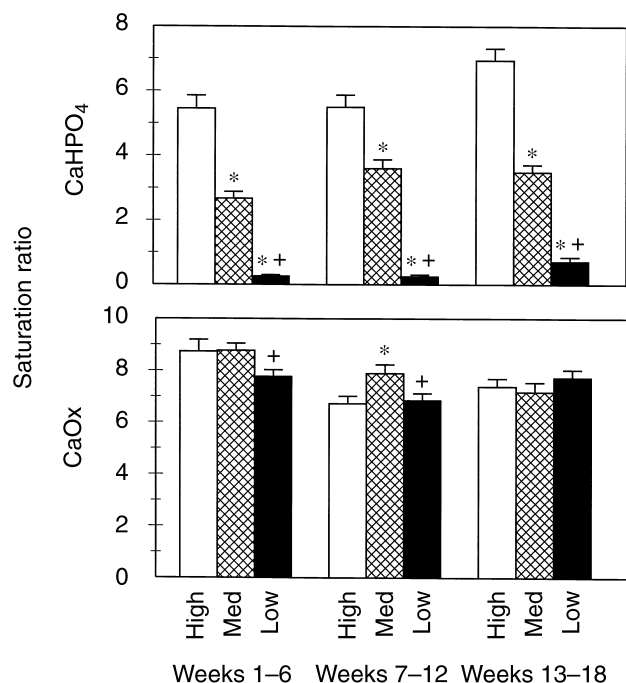


Fig. 4. Relative saturation ratio of brushite (CaHPO_4) and calcium oxalate (CaOx) in GHS rats fed 13 g/day of either a high-, medium-, or low-phosphorus diet for a total of 18 weeks. The methods and abbreviations are the same as in Figure 1.

supporting the role of dietary phosphorus intake altering not only urine phosphorus excretion, but urine calcium excretion as well (Fig. 7). In spite of the increased urine calcium excretion, with no change in oxalate excretion, there was little change in supersaturation with respect to the calcium oxalate solid phase. The lack of change in calcium oxalate supersaturation may be due to a large surplus of urine calcium on each of the three diets, relative to the constant amount of urine oxalate (Fig. 1, middle and bottom). Increasing urine ionized calcium leads to a reciprocal decrease in urine ionized oxalate via the formation of soluble calcium oxalate complexes, resulting in little net change in the product of ionized calcium and ionized oxalate, the determinant of calcium oxalate supersaturation. In this animal model, the ratio of calcium to oxalate is so high that much of the oxalate may actually be incorporated into the soluble dicalcium oxalate species [44, 45].

Similar to calcium, dietary phosphorus complexes to dietary magnesium [46]. In our study, at the lower levels of dietary phosphorus intake there was an increase in urine magnesium excretion. Urine pH and urine citrate tended to fall and ammonium increase at the lowest levels of dietary phosphorus intake. This is not unexpected as the fall in urine phosphorus would make less phosphorus available to bind protons resulting in less titratable acidity and a more acidic urine, which would stimulate renal ammonium production and excretion. In

addition, replacing diet phosphate with chloride has the net effect of providing dietary calcium as CaCl_2 . The presentation of calcium with chloride, rather than an organic anion, results in intestinal chloride for bicarbonate exchange with subsequent lowering of urinary pH [47]. The fall in urine pH during the latter two time periods would contribute to the fall in urine supersaturation with respect to brushite because it would favor the protonation of HPO_4^- to H_2PO_4^- , reducing the concentration of HPO_4^- available to bind with calcium.

The decrease in urine phosphorus excretion, in spite of an increase in urine calcium excretion, led to a marked fall in CaHPO_4 supersaturation. Kidney stones formed in all rats fed the high-phosphorus diet and none of the rats fed the two lower phosphorus diets. In this study, the high-phosphorus diet led to a CaHPO_4 supersaturation ratio of greater than 5. Stones did not form when the ratio was slightly less than 4, suggesting that the critical supersaturation ratio for stone formation is somewhere between 4 and 5. Previously, we showed that stones formed in all rats when the CaHPO_4 supersaturation ratio was also approximately 4, thus confirming our current observations [15]. It is unclear why such a high supersaturation ratio is necessary for stone formation. Possibilities include the presence of inhibitors of stone formation, such as calgranulin [48], nephrocalcin [49], and Tamm Horsfall protein [50], which would retard stone formation *in vivo*. Citrate chelates urinary calcium and is reduced in some patients with nephrolithiasis [1]. However, in this study, the small, but significant reduction in urine citrate in the GHS rats fed the low-phosphate diet was not associated with stone formation. Further studies will be necessary to investigate the role of inhibitors in stone formation in the GHS rats.

Approximately 5 to 10% of kidney stones in humans are predominantly composed of calcium and phosphate [51]. A large proportion of these patients have a renal acidification disorder, such as complete or incomplete distal renal tubular acidosis, resulting in an increased urinary pH. In these patients, there is not only a direct effect of acidosis to decrease renal calcium reabsorption [52], but a component of acidosis induced bone resorption [53], resulting in hypercalciuria [54]. The elevated pH increases the concentration of monohydrogen phosphate, which in addition to the hypercalciuria, increases the supersaturation of calcium phosphate salts. Alkali treatment is conventionally used to correct the systemic acidity, reduce bone resorption, reduce the hypercalciuria and reduce renal stone formation [2]. Alkali treatment will also increase urinary citrate excretion, a known inhibitor of stone formation [55]. However, the increased urinary pH could promote calcium phosphate stone formation [2]. There are no controlled clinical studies to determine whether systemic alkalization is effective in reducing renal stone formation in these patients. Other

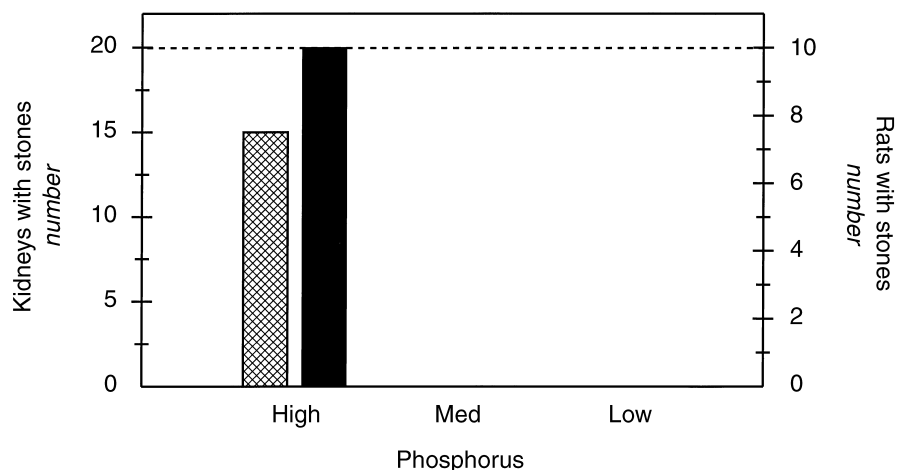


Fig. 5. Number of kidneys with stones (⊗) and number of rats who formed stones (■). GHS rats were fed 13 g/day of either a high-, medium-, or low-phosphorus diet for a total of 18 weeks. Abbreviations are as follows: High, GHS rats fed 13 g/day of a 0.565% phosphorus diet, $N = 10$; Med, GHS rats fed 13 g/day of a 0.395% phosphorus diet, $N = 10$; Low, GHS rats fed 13 g/day of a 0.225% phosphorus diet, $N = 10$; dashed line, maximum number of kidneys or rats with stones.



Fig. 6. Radiograph of stone formation in a representative GHS rat demonstrating nephrolithiasis in both kidneys. Radiographs were obtained by placing the dissected kidneys, ureters, and bladder on radiographic film, and no contrast material was used.

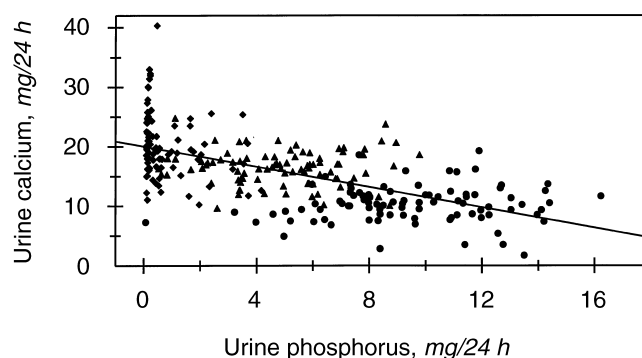


Fig. 7. Urine calcium excretion as a function of urine phosphorus excretion in the GHS rats fed 13 g/day of either a high-, medium-, or low-phosphorus diet for a total of 18 weeks. Every two weeks, two successive individual 24-hour urine collections were obtained and analyzed on the 30 rats divided equally into the three groups. Symbols are (●) GHS rats fed 13 g/day of a 0.565% phosphorus diet; (▲) rats fed 13 g/day of a 0.395% phosphorus diet; (◆) GHS rats fed 13 g/day of a 0.225% phosphorus diet ($r = -0.822$, $n = 289$, $P < 0.001$).

therapeutic modalities such as thiazide diuretic treatment to reduce hypercalciuria or reduction of dietary phosphorus to reduce urinary phosphorus excretion have not been studied. This study, performed in nonacidemic hypercalciuric rats suggests that phosphorus restriction might be beneficial in preventing stones in patients, such as those with renal tubular acidosis, who develop calcium phosphate nephrolithiasis. Controlled studies in patients who form calcium phosphate stones are indicated before this therapy can be recommended.

Thus, we have found that stone formation in GHS rats is critically dependent on dietary phosphorus intake and the resulting change in urinary phosphorus excretion and calcium phosphate supersaturation. A reduction in urinary supersaturation leads to an amelioration of stone formation in this rat model of idiopathic hypercalciuria. Further studies in which humans with renal tubular acidosis or idiopathic hypercalciuria are given graded

amounts of phosphate, their supersaturation determined, and their rate of stone formation documented are necessary to determine if the results of this short-term animal study are applicable to humans.

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